Capacity of simultaneous removal of zinc and cadmium from contaminated media, by two microalgae isolated from a polluted site

Cristina M. Monteiro · Paula M. L. Castro · F. Xavier Malcata

Abstract Several aquatic environments have been contaminated with heavy metals dumped via industrial effluents. Numerous studies have been published regarding the removal of single metals from aqueous solutions by microalgal biomass. However, such studies do not reflect the actual problem associated with industrial effluents because usually more than one metal species is present. Here we studied the biosorption capacity of Zn^{2+} and Cd^{2+} as single- and binary-metal systems by two microalgae, *Scenedesmus obliquus* and *Desmodesmus pleiomorphus*, isolated from a polluted site in Northern Portugal. For each metal independently, *D. pleiomorphus* showed a higher metal sorption capacity than *S. obliquus*, at concentrations ranging from 60 to 300 mg/l (except 150 mg_{Cd}/l). Maximum amounts of Zn^{2+} and Cd^{2+} removed were 22.3 and 60.8 mg/g by *S. obliquus*, and 83.1 and 58.6 mg/g by *D. pleiomorphus*. In binary-metal solutions, *S. obliquus* was in general able to remove Zn^{2+} to higher extents than Cd^{2+}, whereas the opposite was observed with *D. pleiomorphus*. The simultaneous uptake of Zn^{2+} and Cd^{2+} by both microalgae was considerably lower than that of their single-metal counterparts, at equivalent concentrations. Although microalgal uptake from binary-metal solutions was lower than from single-metal ones, the wild microalgae selected were able to efficiently take up mixtures of Zn^{2+} and Cd^{2+} up to 300 mg/l of both metals—thus materializing a promising bioremediation vector for polluted waters.

Keywords *Scenedesmus obliquus* · *Desmodesmus pleiomorphus* · Simultaneous biosorption · Binary mixture · Heavy metals · Zn and Cd

C. M. Monteiro · P. M. L. Castro
CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

F. X. Malcata (✉)
ISMAI, Instituto Superior da Maia, Avenida Carlos Oliveira Campos, Castelo da Maia, 4475-690 Avioso S. Pedro, Portugal
e-mail: fmalcata@ismai.pt

F. X. Malcata
CIMAR/CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas no. 289, 4050-123 Porto, Portugal

Introduction

Pollution of aquatic environments by toxic heavy metals has been taking place because of discharge of untreated effluents from many industrial processes. The environmental injuries brought about by such effluents have received major attention by national and international authorities, and consequently led to directives and regulations aimed at minimizing their impact. In attempts to remove (or, at least, reduce the concentration of) those toxic metals, distinct types of microbial biomass have been scrutinized as alternatives to conventional physicochemical technologies (Aksu and Dönmez 2006; Puranik and Paknikar 1999; Sağ et al. 2000; Vilar et al. 2008); the latter are in fact characterized by a limited effectiveness, and are typically too expensive when the target metal concentration is at the ppm level or below (Fraile et al. 2005).

One type of biomass useful for bioremediation is that from microalgae. The capacity of these microorganisms to remove heavy metals has mainly been attributed to metal adsorption onto their cell wall, by both electrostatic attraction and complexation (Aksu and Dönmez 2006; Mehta and Gaur 2005). The cell wall of microalgal cells

includes indeed several macromolecules that act as polyelectrolytes owing to their charged side groups, and thus provide binding sites for the aforementioned metal ions (Mehta and Gaur 2005; Puranik and Paknikar 1999); the presence of anionic and cationic sites brings about amphoteric properties for the cell wall.

Most experimental data available in the literature pertain, however, to single-metal biosorption by microalgae; multi-metal systems have received a poor attention by researchers, maybe because of their intrinsically higher complexity arising from the possible interactions among solutes. However, wastewaters normally contain more than one type of metal ion, so each metal may interfere with removal and/or recovery of other metal(s) of interest (Aksu and Dönmez 2006; Puranik and Paknikar 1999). It is known that the extent of metal removal depends on intrinsic factors, such as specific surface properties of the microorganism, and extrinsic factors, such as temperature, pH, metal ion concentration and biomass concentration (Aksu and Dönmez 2006; Mehta and Gaur 2005). Furthermore, the presence of other cations may hamper sorption of a given metal by biomass: the capacity of microorganisms to bind more than one metal species depends, in fact, on several extra parameters, e.g. metal ion combination, metal ion concentrations and order of metal addition (Ting et al. 1991).

Competition among the various metal ions in solution for microalga surface binding sites will likely occur, depending on their specific features as cations (Aksu and Dönmez 2006; Aksu et al. 1999). A mixture of metal ions can typically produce three types of behaviour: synergism, antagonism or no interaction at all; however, which specific behaviour will take place cannot be anticipated solely on the basis of single-metal studies (Aksu et al. 1999; Puranik and Paknikar 1999). Consequently, a practical need exists for studying the removal process in multiple-metal solutions, at least binary ones.

The objective of the present study was thus to assess the performance of two wild microalgae in removal of Zn^{2+} and Cd^{2+} from aqueous solutions of single- and binary-metal systems—and to determine the combined effects of these metal ions on the bioaccumulation capacity of *S. obliquus* and *D. pleiomorphus*.

Experimental procedure

Microalga sources and culture conditions

The test microalgae, *Scenedesmus obliquus* and *Desmodesmus pleiomorphus*, were isolated from a heavy-metal-contaminated region of Northern Portugal, "Esteiro de Estarreja" (Monteiro et al. unpublished), where Zn appears as a major contaminant (Oliveira et al. 2001); hence, their intrinsic capacity for metal uptake was expected to be high enough for eventual bioremediation on larger scale. Both microalga species were cultured in OHM medium (Bishop and Senger 1971) in the absence of EDTA, and were maintained at 25°C under continuous lighting. Inocula for all experimental batches were obtained from exponentially growing cultures, based on cell numbers (this status was reached after 2–3 days of growth). All materials and culture media were previously autoclaved at 121°C and 1 atm, for 15 min.

Biosorption experiments

Stock solutions of Zn^{2+} (5.0 g/l) and Cd^{2+} (6.0 g/l) were prepared with the corresponding chloride salts, $ZnCl_2$ and $CdCl_2$, dissolved in deionized water. All glassware material employed was previously rinsed with nitric acid and several times afterwards with deionized water prior to use, so as to guarantee absence of any interferent with the chemical analyses afterwards.

The capacity of each of the two microalga species to remove Zn^{2+} and Cd^{2+}, both as individual entities and as a mixture, was examined in batch cultures stirred at 100 rpm and kept at 25°C. The biosorption experiments were performed in triplicate, in glass flasks containing 25 ml of the metal aqueous solutions with the intended concentrations of a single or of the two cations. A defined volume of microalgal culture was then added to the experimental media, in order to achieve an initial cell density of ca. 1 g/l dry weight (more specifically, 0.8 g/l for *S. obliquus* and 1.0 g/l for *D. pleiomorphus*).

After exposure of the microalgal cells to metal solutions for a period of 2 h, 15-ml (duplicated) samples were taken from each flask. They were subsequently centrifuged, and the supernatant was collected for determination of the remaining concentrations of both Zn^{2+} and Cd^{2+} in the culture medium by atomic absorption spectrophotometry with flame atomization, using an Unicam 960 (USA) spectrophotometer, at the wavelengths of 213.9 and 228.8 nm for Zn and Cd, respectively. Based on previous experience entailing Zn^{2+} and Cd^{2+} removal by either microalgal species (Monteiro et al. 2009; 2010a), a 2-h exposure period was chosen for these sorption studies; it had indeed been observed that removal of metal cations by those microalgae is mainly (but not exclusively) by adsorption onto their surface—which is a rapid process that reaches equilibrium after a few minutes of contact. The initial concentrations used were 60, 150 and 300 mg/l in single-metal tests, and 60 mg_{Zn}/l + 60 mg_{Cd}/l, 60 mg_{Zn}/l + 300 mg_{Cd}/l, 150 mg_{Zn}/l + 150 mg_{Cd}/l, 300 mg_{Zn}/l + 60 mg_{Cd}/l and 300 mg_{Zn}/l + 300 mg_{Cd}/l in combined-metal tests. The combinations of Zn^{2+} and Cd^{2+} in binary

solutions were based on a factorial scheme coupled with a sequential dilution strategy. Negative controls (i.e. culture media with the desired metal concentrations, but without microalgal biomass) were considered as well.

Results and discussion

The capacity of Zn^{2+} and Cd^{2+} removal by *S. obliquus* and *D. pleiomorphus*, two microalga species isolated from a contaminated location in Northern Portugal, were tested in both single and binary-metal systems. Experimental data on metal removed using the two microalgae, expressed per unit weight of biomass, as well as individual and total removal efficiencies, are presented in Fig. 1, and in Tables 1 and 2. Although more concentration levels could have been in principle tested, the number of experiments to be run under a factorial design would have become unpractical, especially because of the combinations of both metals, so only a 3-point design was pursued.

It has been claimed that the removal capacity of living biomass occurs both by adsorption onto the cell surface and by incorporation inside the cell. However, previous studies have shown that, in the case of the microalgae selected for our study, both Zn^{2+} and Cd^{2+} independently were removed chiefly by adsorption onto the cell surface (Monteiro et al. 2010a, b). Although non-living microalgae would thus logically appear as more appropriate, note that passive removal is anyway lower than total removal, and that decay of the adsorbent would be relatively fast if inactivated biomass were employed. On the other hand, the capacity of living biomass to remove heavy metals from solution is likely affected by the toxicity of said heavy metal pollutants, as they cause changes in cell metabolism and growth (Omar 2002); previous studies encompassing Zn^{2+} and Cd^{2+} toxicity upon either *S. obliquus* or *D. pleiomorphus* cells (Monteiro et al. 2010c) have indeed demonstrated that growth is significantly affected by the presence of any of those metals, but only at relatively high concentrations—i.e. above the upper bound considered here for the initial heavy metal concentrations. Therefore, resorting to living biomass was a reasonable option.

In Fig. 1, one observes that the amount of Zn^{2+} accumulated by *D. pleiomorphus* increased with increasing initial metal ion concentration in single-metal ion experiments; regarding exposure to plain Cd^{2+}, an increase in

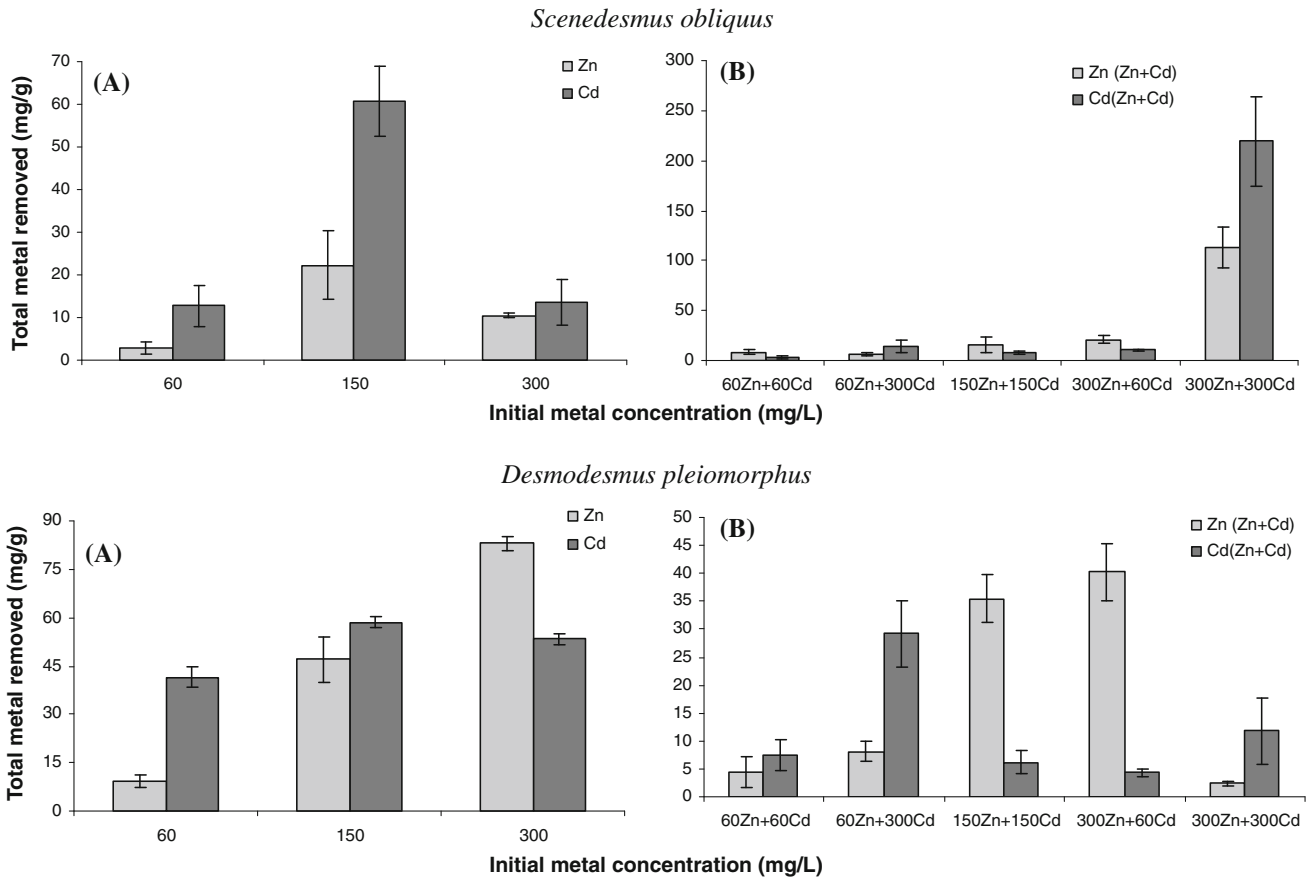

Fig. 1 Extents of Zn and Cd removed (average ± standard deviation) from single (**a**) and binary (**b**) metal aqueous solutions, by *S. obliquus* and *D. pleiomorphus*

Table 1 Extents of Zn^{2+} and Cd^{2+} removed (average ± standard deviation) by *S. obliquus* at various initial metal concentrations, from single- and binary-metal aqueous solutions, and corresponding bioaccumulation efficiencies

Initial Zn concentration (mg/l)	Initial Cd concentration (mg/l)	Total Zn removed (mg/l)[a]	Total Cd removed (mg/l)[a]	Zn removal efficiency $-Y_j$ (%)[b]	Cd removal efficiency $-Y_j$ (%)[b]	Combined removal Efficiency $-Y_{total}$ (Zn + Cd) (%)[c]
60	0	2.3 ± 1.1	–	3.9	–	3.9
150	0	17.8 ± 6.4	–	11.9	–	11.9
300	0	8.3 ± 0.4	–	2.8	–	2.8
0	60	–	10.2 ± 3.8	–	16.9	16.9
0	150	–	48.6 ± 6.6	–	32.4	32.4
0	300	–	10.8 ± 4.3	–	3.6	3.6
60	60	6.8 ± 1.7	2.9 ± 1.1	11.2	4.8	8.1
60	300	4.9 ± 1.2	11.1 ± 4.7	8.1	3.7	4.4
150	150	12.3 ± 6.3	6.0 ± 1.4	8.2	4.0	6.1
300	60	16.7 ± 3.0	8.3 ± 0.9	5.6	13.8	7.0
300	300	90.5 ± 16.3	175.6 ± 3.6	30.2	58.5	44.3

[a] Total metal removal (Metal removed(mg/l) = [Initial Metal Ion] − [Metal Ion in Solution at Equilibrium])

[b] Single-metal removal efficiency $\left(Y_j = \frac{[\text{Metal Ion Removed}]\,(\text{mg/l})}{[\text{Initial Metal Ion}]\,(\text{mg/l})} \times 100\right)$

[c] Total removal efficiency $\left(Y_{\text{Total}} = \frac{\sum [\text{Zn + Cd Total Metal Ion Removed}]\,(\text{mg/l})}{[\text{Initial Cd + Zn Metal Ion}]} \times 100\right)$

Table 2 Extents of Zn^{2+} and Cd^{2+} removed (average ± standard deviation) by *D. pleiomorphus* at various initial metal concentrations, from single- and binary-metal aqueous solutions, and corresponding bioaccumulation efficiencies

Initial Zn concentration (mg/l)	Initial Cd concentration (mg/l)	Total Zn removed (mg/l)[a]	Total Cd removed (mg/l)[a]	Zn removal efficiency $-Y_j$ (%)[b]	Cd removal efficiency $-Y_j$ (%)[b]	Combined removal Efficiency $-Y_{\text{total}}$ (Zn + Cd) (%)[c]
60	0	9.4 ± 1.9	–	15.7	–	15.7
150	0	47.1 ± 7.0	–	31.4	–	31.4
300	0	83.1 ± 2.1	–	27.7	–	27.7
0	60	–	41.4 ± 3.2	–	69.0	69.0
0	150	–	58.6 ± 1.7	–	39.1	39.1
0	300	–	53.3 ± 1.8	–	17.8	17.8
60	60	4.4 ± 2.7	7.4 ± 2.8	7.3	12.3	9.8
60	300	8.1 ± 1.8	29.2 ± 5.9	13.5	9.7	10.4
150	150	35.4 ± 4.3	6.2 ± 2.1	23.6	4.1	13.9
300	60	40.2 ± 5.2	4.3 ± 0.6	13.4	7.2	12.4
300	300	2.4 ± 0.4	11.8 ± 6.0	0.8	3.9	2.4

[a] Total metal removal (Metal removed(mg/l) = [Initial Metal Ion] − [Metal Ion in Solution at Equilibrium])

[b] Single-metal removal efficiency $\left(Y_j = \frac{[\text{Metal Ion Removed}](\text{mg/l})}{[\text{Initial Metal Ion}](\text{mg/l})} \times 100\right)$

[c] Total removal efficiency $\left(Y_{Total} = \frac{\sum [\text{Zn + Cd Total Metal Ion Removed}](\text{mg/l})}{[\text{Initial Cd + Zn Metal Ion}]} \times 100\right)$

metal concentration only produced an increase in the amount removed from 60 to 150 mg/l (between 150 and 300 mg_{Cd}/l, similar amounts were nevertheless observed); the same trend of an increasing bioaccumulation capacity of *S. obliquus* was observed for both metals when the initial concentration increased from 60 to 150 mg/l; on the other hand, a significant decrease was noticed after exposure to the highest concentration tested (i.e. 300 mg/l). Since the metal was removed mainly by adsorption onto the cell surface, this pattern could be rationalized by a release of

metal ions from the cell wall as a defence mechanism developed by viable microalgae against the toxic effects of heavy metals at higher concentrations. Comparing the adsorption capacity of both species in single-metal solutions, *D. pleiomorphus* outperformed *S. obliquus* except at 150 mg_{Cd}/l. Furthermore, taking into account the amount of both Zn^{2+} and Cd^{2+} removed by each microalga, the adsorption capacity of Zn^{2+} was typically lower than that of Cd^{2+} (except for *D. pleiomorphus* exposed to 300 mg_{Zn}/l). The highest levels of Zn^{2+} and Cd^{2+} uptaken by *S. obliquus* were 22.3 ± 8.1 and 60.8 ± 8.2 mg/g, respectively, when exposure was to an initial concentration of 150 mg/l; the maximum amounts of Zn^{2+} and Cd^{2+} uptaken by *D. pleiomorphus* were 83.1 ± 2.1 and 58.6 ± 1.7 mg/g, via exposure to initial metal concentrations of 300 and 150 mg/l, respectively.

The simultaneous removal of Zn^{2+} and Cd^{2+} from binary mixtures was then compared with the removal amounts obtained in single-metal solutions. For *D. pleiomorphus*, the amounts of Zn^{2+} removed in the presence of increasing Cd^{2+} concentrations and vice versa (i.e. the amounts of Cd^{2+} uptaken in the presence of increasing Zn^{2+} concentrations), in combined-metal tests, were lower than those of the corresponding single-metal system—thus indicating lower removal efficiencies (Fig. 1 and Table 2); for instance, the maximum Zn^{2+} uptake in the latter case was 83.1 ± 2.1 mg/g after exposure to 300 mg/l, but such an uptake decreased to 40.2 ± 5.2 and 2.4 ± 0.4 mg/g when Cd^{2+} was initially present at 60 and 300 mg/l, respectively—under an identical concentration of Zn^{2+}.

The amount of Zn^{2+} uptaken by *S. obliquus* in the binary-metal system was higher than in its single-metal counterpart, except at the initial concentrations of 150 mg/l of Zn^{2+} and Cd^{2+} (Fig. 1 and Table 1). Conversely, the amounts of Cd^{2+} removed were generally lower when compared with their single-metal solutions (except at 300 mg/l as initial concentration of both metals). Moreover, the amounts of Zn^{2+} uptaken in this type of solutions were generally higher than those of Cd^{2+} (except at 60 mg_{Zn}/l + 300 mg_{Cd}/l, and 300 mg/l of both metals). When in the presence of both Zn^{2+} and Cd^{2+}, *S. obliquus* revealed an adsorption capacity of 219.5 mg_{Cd}/g after exposure to 300 mg/l of both metals—thus showing a higher affinity for Cd ions when the maximum removal extent was achieved.

It was noticed that, in the presence of binary-metal solutions, *S. obliquus* produced in general a higher removal of Zn^{2+} than Cd^{2+} for the combination of concentrations tested, except when Cd^{2+} was present at 300 mg/l, which led to twice as much Cd^{2+} removed; hence, a preference towards removal of Zn^{2+} was observed for this microalga when exposed to the other metal. However, an opposite tendency was revealed by *D. pleiomorphus*—a preference for Cd ions in binary-metal solutions was indeed observed. Sánchez et al. (1999) reported that, in binary systems encompassing Cu and Cd, Cu was preferentially adsorbed by *Cymodocea nodosa*. Similarly, Puranik and Paknikar (1999) described preferential uptake of Pb in binary-metal systems (e.g. Pb^{2+}+Cd^{2+} and Pb^{2+}+Zn^{2+}, among others).

In general, three types of interactive sorption behaviour are displayed by a mixture of heavy metals—viz. synergism, antagonism or lack of interaction. In the former case, the effect of the mixture is greater than the sum of the individual effects of its constituents. In the case of antagonism, the effect of the mixture is less than the sum of the individual effects of the constituents thereof. When no interaction is present, the effect of the mixture is equivalent to the sum of the individual effects (Qi and Aldrich 2008). To determine whether those cation combinations, for either microalga species, interacted in a synergistic or antagonistic manner (or none of them), the removal efficiencies in single- and double-metal ion systems were compared to each other. For instance, using the data tabulated in Table 1 referring to *S. obliquus*, one would expect that the total removal efficiency in the mixture containing 60 mg/l of each metal should equal 16.0% (i.e. 11.2 + 4.8%) for a total 120 mg/l of both metals; however, the observed total removal efficiency was only 8.1%, which represents ca. one half of the total. Consequently, the effect of a second metal upon uptake was found to be unfavourable in terms of total metal removal, although an increase could be noticed in the amounts of Zn^{2+} removal after addition of Cd^{2+} in all metal combinations (except at 150 mg/l of both metals); hence, the interaction between Zn^{2+} and Cd^{2+} should be labelled as antagonistic. A similar trend was recorded for *D. pleiomorphus*.

Recall that the cell wall of microalgal cells is composed of several macromolecules (e.g. peptidoglycan, polysaccharides and proteins) that carry such charged groups as carboxyl, phosphate, hydroxyl or amine, and thus provide binding sites for metal ions (Mehta and Gaur 2005; Puranik and Paknikar 1999). Depending on the prevailing pH, such groups may be either protonated or deprotonated, which obviously affects the extents of metal removal from the surrounding media (Aksu and Dönmez 2006). The most logical reason for the aforementioned antagonistic action would be a similar biosorption mechanism and thus a competitive demand for binding sites on the surface of the microalga cells, associated with mutual exclusion of one metal by the other, owing to stereochemical hindrance (which is expected to be stronger for similar cations). Since a fixed-cell biomass offers a finite number of surface binding sites, the equilibrium uptake would be expected to attain saturation at high total metal ion concentrations, even if the individual concentrations would not cause saturation *per se*; this does not result only from saturation of

the biomass, but is also related to interferences between concentrated components that compete for partially overlapped sorption sites.

Aksu and Dönmez (2006) described that biosorption of Cd^{2+} by the biomass of *Chlorella vulgaris* (which has cell wall structure and charge similar to those of the microalga species tested in this study) was preferential to that of Ni^{2+} in binary systems, thus showing a greater sorption capacity for Cd^{2+} than Ni^{2+}. The inhibitory effect brought about by the presence of another cation in solution may be somehow predicted on the basis of the difference in their class behaviour, as suggested by Nieboer and McBryde (1973). Metal ions can accordingly be grouped into class "a", "b" or borderline ions, on the basis of their covalent indices; both metals used in our study are borderline ions, and Tsezos et al. (1996) claimed that ionic competition is greater (and thus more unfavourable) between metals belonging to the same class. Studies reported by Fraile et al. (2005) encompassing the simultaneous removal of Zn^{2+} and Cd^{2+} also by the microalga *C. vulgaris* encompassed all possible binary-metal combinations using 0, 10, 20, 30 and 250 mg/l as initial metal concentrations; they showed that the presence of Cd^{2+} produced a sharp decrease in Zn^{2+} sorption capacity, so there appears to be a clear affinity of biomass for Cd^{2+} in the binary system Cd^{2+}-Zn^{2+}.

However, there is a serious lack of data published regarding metal removal from binary solutions using microalgae; hence, data reported in the literature pertaining to other (micro)organisms were chosen for putative comparison. A similar antagonistic effect was found by Açıkel and Alp (2009) for the fungus *Rhizopus delemar*, when bioaccumulation of Cu^{2+} and Ni^{2+} was reduced by the presence of increasing concentrations of the second metal ion—as compared with single-metal systems. Tobin et al. (1988) pointed out that direct competition appears to exist regarding uptake of Zn^{2+}–Cd^{2+} by *Rhizopus arrhizus*. Mehta and Gaur (2001) reported that Ni^{2+} and Cu^{2+} inhibited biosorption of each other, with a competition mechanism involving the same non-specific binding sites. Sheng et al. (2007) reported as well that the uptake capacity of the alga *Sargassum* sp. for Pb was reduced in the presence of Cu and Cd, and that the inhibitory effect of the competing metal ions increased as the concentration of each of those metal ions increased in solution.

In our study, the microalga *D. pleiomorphus* exhibited a net preference for Cd over Zn ions, whereas *S. obliquus* behaved the opposite fashion in binary-metal solutions. Those observations are consistent with other studies: Yu and Kaewsarn (1999) and Mallick (2003) described a lower uptake of Cu^{2+}–Cd^{2+} and Ni–Cu by *Durvillaea potatorum* and *C. vulgaris*, respectively, whereas Aksu et al. (1997), regarding Fe^{3+} and Cr^{6+} binary systems, found that the biosorption yields by *C. vulgaris* were reduced faster and faster by the presence of increasing concentrations of the other metal, thus claiming an antagonistic behaviour.

Overall, one realizes that several studies have been performed to test the metal removal capacity of microalgal biomass, but that only a few have used microalgal species isolated from contaminated locations – which have obviously proven to lead to a higher removal capacity than by species obtained from non-polluted environments. On the other hand, only scarce studies have been conducted to test the ability of microalgae to remove metals from multi-metal solutions, which are more similar to the effluents found in common practice. Since mixtures of Zn^{2+} and Cd^{2+} are typically found in contaminated effluents of industrial processes (Alloway and Ayres 1997), testing of combinations of these two metals would be in order. In this study, it was demonstrated that the two wild microalgae tested are suitable for bioremediation strategies, as both showed a good removal capacity of the two heavy metals. However, there is a competition between the two metals that causes a decrease in the observed overall removal capacity of the metals in the presence of each other, when compared with the single-metal counterparts.

Conclusions

Microalgal biomass has shown the ability to remove heavy metals from solution, in both single and binary systems. Solutions of two (or more) heavy metal species may experience synergistic or antagonistic interactions with regard to individual metal uptake by microalgae; since mixtures of Zn^{2+} and Cd^{2+} are typically found in contaminated effluents of industrial processes, the combined removal capacity should be tested as it better parallels actual industrial effluents.

The adsorption capacity of Zn^{2+} by *S. obliquus*, when in the presence of Cd^{2+}, is higher than that of Zn^{2+} alone; hence, Zn^{2+} was adsorbed preferentially over Cd^{2+}. An opposite trend was observed with *D. pleiomorphus*, for which a preference for Cd ions in binary-metal solutions was unfolded. Nevertheless, the main finding in this work was that the overall uptake capacity of either microalga species in binary-metal solutions is lower than in the corresponding single-metal systems under similar conditions; hence, competitive adsorption between the two ions appears to exist, which might even be of an antagonistic nature. This point is of major practical interest, since the bioremediation capacity of a microalga regarding a given heavy metal ion may be overestimated if other metal ion(s) is(are) present—which is a more likely scenario when dealing with industrial effluents. Therefore, this research effort constituted a first step towards development of a large-scale wastewater treatment process using microalgae.

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